HAYATI Journal of Biosciences 24 (2017) 46–52

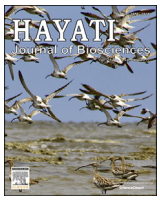
HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences



Original research article

Endophytic Fungi Associated With Turmeric (*Curcuma longa* L.) Can Inhibit Histamine-Forming Bacteria in Fish

Eris Septiana,[1,3] Nampiah Sukarno,[1*] Sukarno,[2] Partomuan Simanjuntak[3]

[1] *Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Darmaga Campus, Bogor, Indonesia.*
[2] *Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, Darmaga Campus, Bogor, Indonesia.*
[3] *Research Center for Biotechnology, Indonesian Institute of Sciences, Jalan Raya Bogor Km 46, Cibinong, Bogor, Indonesia.*

ARTICLE INFO

Article history:
Received 24 July 2016
Accepted 11 May 2017
Available online 13 June 2017

KEYWORDS:
antimicrobial activity,
medicinal plant,
Morganella morganii,
phylogenetic analysis,
ribosomal DNA

ABSTRACT

Turmeric (*Curcuma longa* L.) is a medicinal plant that is commonly used as spice and preservative. Many types of endophytic fungi have been reported as being associated with medicinal plants and able to synthesize secondary metabolites. In this study, endophytic fungi were isolated from all plant parts of turmeric plants. Identification of the endophytic fungi was done using morphological characteristics and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA. The dual culture method was used for screening antibacterial activity of the endophytic fungi against *Morganella morganii*, a common histamine-producing bacteria. The disc diffusion method was used to test the ability of water fractions of selected endophytic fungi to inhibit *M. morganii* growth. Two-dimensional thin layer chromatography was used to determine the fungal extract inhibition activity on histamine formation. In total, 11 endophytic fungi were successfully isolated and identified as *Arthrobotrys foliicola*, *Cochliobolus kusanoi*, *Daldinia eschscholzii*, *Fusarium oxysporum*, *Fusarium proliferatum*, *Fusarium solani*, *Fusarium verticillioides*, *Phanerochaete chrysosporium*, and *Phaeosphaeria ammophilae*. Five isolates showed inhibition activity against *M. morganii* in the dual culture tests. Based on the disc diffusion assay, *A. foliicola* and *F. verticillioides* inhibited the growth of *M. morganii* as a histamine-producing bacteria, and inhibiting histamine formation in fish. The best effects in inhibiting growth of the histamine-producing bacteria and histamine formation inhibition in fish were produced with *F. verticillioides* water fraction at 0°C incubation.

1. Introduction

Turmeric (*Curcuma longa* L.) is a zingiberacean plant that grows in the Southeast and South Asia. In Indonesian society, turmeric is used for cooking, natural food dyes, and as a remedy for various diseases. Either on its own or combined with salt, it is also used as a natural preservative for fresh fish to extend shelf life (Akter *et al.* 2013). It has been reported that medicinal plants are potential sources of endophytic fungi that have the ability to produce bioactive compounds (Ginting *et al.* 2013). Turmeric as a medicinal plant, could be host to various endophytic fungi, which potentially have either the same or different biological activities as their host (Zhao *et al.* 2011). Endophytic fungi are fungi that live inside plant tissues and perform a beneficial function for their host, through production of bioactive compounds that protect the host from biotic and abiotic stress (Dai *et al.* 2008). In return, the host provides a unique ecological niche for growth and development of the endophytic fungi (Barrow *et al.* 2008).

Histamine is a chemical compound normally produced by the decarboxylation of free histidine by the L-histidine decarboxylase enzyme, which is synthesized by histamine-producing bacteria. These bacteria are often found in improperly preserved scombroid fish (Bjornsdottir *et al.* 2009). Under normal conditions, eating fish containing small quantities of histamine will have little negative effect on the health. However, in larger amounts, consumption may cause histamine poisoning, whose effect could include diarrhea, headache, hypotension, pruritus, and flushes (Wohrl *et al.* 2004). Therefore, histamine levels need to be controlled to reduce cases of histamine poisoning, especially after consuming fish containing histamine.

* Corresponding author.
E-mail address: ipi.sukarno@gmail.com (N. Sukarno).
Peer review under responsibility of Institut Pertanian Bogor.

http://dx.doi.org/10.1016/j.hjb.2017.05.004

Several studies have been performed on turmeric extract as a growth inhibitor of histamine-producing bacteria. Paramasivam *et al.* (2007) reported that a 5% concentration of turmeric extract inhibited the growth of histamine-producing bacteria, *Morganella morganii* also inhibited the formation of histamine. Previous studies on growth inhibition of histamine-producing bacteria in fresh fish in Indonesia have been carried out only in respect of the effect of bioactive compounds derived from turmeric plant. There has been no report on the uses of endophytic fungi isolated from turmeric plant, specifically, to inhibit the growth of histamine-producing bacteria and thus inhibit histamine formation in fish. Studies on the application of endophytic fungi associated with the turmeric plant should provide information on the potential of these fungi as an alternative natural product for reducing histamine levels in fish. Therefore, this research was carried out to study the endophytic fungi associated with the turmeric plant, and to determine their inhibition effects on the growth of the histamine-producing bacteria, *M. morganii*, and on the formation of histamine in fresh fish.

2. Materials and Methods

2.1. Isolation and identification of endophytic fungi

Turmeric plants were obtained from Tanah Sareal District, Bogor, West Java. The plant organs used for isolation were the root, rhizome, stem, leaf, inflorescence, and flower. Isolation of endophytic fungi was carried out using the same process described by Hallmann *et al.* (2006). The samples were washed with water to remove dirt, and then dried at room temperature using sterile filter paper. Each piece of the plant organ was cut into 2 cm long segments for the roots, rhizomes, and stems, or 2 × 2 cm square pieces for leaves, inflorescences, and flowers.

Surface sterilization of each sample was performed by soaking the samples in 70% ethanol for 1 minute, 5.3% sodium hypochlorite for 5 minutes, 70% ethanol for a further 30 seconds and then rinsed three times using sterile distillated water. All samples were dried for 6 hours on sterile filter paper in a safety cabinet. Samples were then further cut into 1 cm long segments for the roots, rhizomes, and stems, whereas leaves, inflorescences, and flowers were further cut into 1 × 1 cm square pieces. Three pieces or segments of each organ were placed in Petri dishes on low carbon agar (LCA) medium containing rose bengal (25 mg/L) as a fungistatic agent and chloramphenicol (250 mg/L) as a suppressor of bacterial growth. Three replicates were prepared for each organ. As a negative control, 1 mL of sterile water from the final rinse was plated on LCA medium and then incubated at the ambient temperature. The hyphae from the fungal colonies were transferred to a fresh LCA medium without rose bengal and chloramphenicol, and incubated at the ambient temperature for 7 days.

Isolated endophytic fungi were identified by morphological characteristics (Barnett and Hunter 1998), and by molecular analysis using ITS1, ITS2, and 5.8 region sequences, using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) as a forward primer and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) as a reverse primer (White *et al.* 1990). For DNA extractions, fungal mycelium was grown in a 50-mL flask containing Potato Dextrose Broth, incubated in 120 rpm shaker at 29°C for 7 days. Mycelia were harvested by filtration with sterile filter paper then washed by sterile distillated water, then ground in a sterile pestle and mortar with the addition of liquid nitrogen. DNA extraction was performed using the cetyltrimethyl ammonium bromide (CTAB) method (Sambrook and Russell 2001).

Polymerase chain reaction (PCR) amplification was performed on a total volume of 30 μL containing 10.5 μL sterile free base water, 15 μL 2× PCR Master Mix (Promega, USA), 0.75 μL 10 pmol of each of ITS1 and ITS4 primers, and 3 μL (~250 ng/μL) of the DNA template. Amplification reaction was performed in 35 cycles as follows: predenaturation at 95°C for 1.5 minutes, denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1.5 minutes, final extension at 72°C for 5 minutes, and then stored at 25°C for 10 minutes. An amount of 5 μL PCR product was analyzed using gel electrophoresis (250 volt; 75 watt) containing 1% agarose for 30 minutes, stained with ethidium bromide for 25 minutes and observed under UV light.

Purification and sequencing of PCR products using the same primers were carried out by a service provider, First BASE (Singapore). The sequences were analyzed using the ChromasPro program (Technelysium, Australia) and determined using available DNA fungal sequences, via searches of the MycoBank database (http://www.mycobank.org) and the BLAST (http://www.blast.ncbi.nlm.nih.gov/blast). Phylogenetic analyses were conducted using the neighbor-joining (N-J) method in MEGA5 (Tamura *et al.* 2011), and an N-J tree was constructed using the Kimura-2 parameter model. All characters were equally weighted and unordered. Gaps and missing data were treated as partial deletions. Support for specific nodes on the N-J tree was estimated using 1000 bootstrapping replications.

2.2. Screening of antibacterial activity by endophytic fungi

Histamine-producing bacteria, *M. morganii* FNCC 0122 for microbial testing were obtained from the Food and Nutrition Culture Collection, PAU Food and Nutrition Gadjah Mada University, Yogyakarta, Indonesia. The bacteria were grown firstly on a modified Niven agar medium to confirm that the bacteria produced histamine. The dual culture method (Zhang *et al.* 2009) was used to screen antibacterial activity. Each pure isolate of 7-day-old endophytic fungi in Petri dishes subsequently were perforated with a hole punch (6 mm in diameter), and placed on a nutrient agar medium containing a 24-hour-old bacterial test culture. The Petri dishes were incubated at the ambient temperature for 24 hours. Clear zone diameters produced around the pieces of endophytic fungi were observed.

Endophytic fungal isolates that produced positive inhibition zones were selected for further assay, to determine their antibacterial activity. The isolates were cultured in 2 L of Potato Dextrose Broth medium in an Erlenmeyer flask, shaken at 120 rpm for 15 days at the ambient temperature. Three replicates were prepared for each isolate. The mycelia were harvested using vacuum filtration to separate culture filtrate and mycelia. The filtrate was extracted using ethyl acetate and partitioned with water to obtain water fraction, whereas the mycelial biomass was extracted using water. The Kirby-Bauer disc diffusion method was used to test the extracts against *M. morganii* FNCC 0122. Each extract with serial concentrations of 10,000, 20,000, and 40,000 ppm was tested against *M. morganii* FNCC 0122 in a Müller-Hinton agar medium. Each treatment was carried out using three replicates. The diameter of the clear zone around the disc after incubation for 24 hours at ambient temperature was measured and recorded. The isolates that produced the highest activity were selected for testing inhibition of histamine formation and of histamine-producing bacterial growth in fresh fish.

2.3. Inhibition test of histamine and histamine-producing bacterial growth in fresh fish

Tests were carried out on tuna fish, which was obtained from fish auctions at traditional markets. The fresh fish was washed with sterile distilled water, and the entrails and skin were cleaned to produce 10 grams pieces of fish fillet. The fillets were divided into two groups, the 1st group was immersed for 30 minutes in the filtrate water fraction of selected endophytic fungi, and the 2nd group in sterile distilled water. The concentration of the filtrate

water fraction used was 40,000 ppm. After immersion, each group was divided into three groups for incubation treatment at either 0, 4, or 29°C for a period of 24-hour incubation. All fish fillet samples were then extracted using methanol. Histamine levels produced by the fillets were measured by two-dimensional thin layer chromatography. The solvents used were mixtures of acetone and ammonia (95:5), and ethanol and ammonia (80:20). Plates were sprayed with 0.1% ninhydrin solution, and heated in an oven at 105°C for 5 minutes (Wortberg and Gerd 1981). Spot of samples formed were compared with the standard spot. Total plate count of histamine-producing bacteria from each fillet was also measured on modified Niven medium. Data analysis was performed using analysis of variance followed by Duncan multiple range test at the 0.05 level for significant difference.

3. Results

3.1. Endophytic fungi associated with the turmeric plant

A total of 11 isolates of endophytic fungi were obtained from the various parts of the turmeric plant: from the rhizomes, roots, flowers, inflorescences, leaves, and stems. The rhizome was inhabited by the highest number of different fungi (four isolates), followed by the flower (two isolates) and the inflorescence (two isolates); and the lowest number was found in the root, leaf, and stem, each of which organs was occupied by only one isolate. Based on morphological characteristics, all 11 endophytic fungal isolates belonged to two isolates of mycelia sterilia and four genera, namely *Arthrobotrys*, *Daldinia*, *Drechslera*, and *Fusarium* (data not shown). There was no fungal growth observed in the negative control treatment.

Based on BLAST analysis, the similarity varied from 81% to 100% (Table 1). The 11 isolates were identified as representing nine species (Table 1 and Figure), belonging to six genera (Table 1). Based on internal transcribed spacer (ITS) region sequences, mycelia sterilia 1 had 93% similarity to *Phaeosphaeria ammophilae*, whereas mycelia sterilia 2 had 99% similarity to *Phanerochaete chrysosporium*. Isolate ClI1 had 81% similarity with *Cochliobolus kusanoi* based on a BLAST search (Table 1). Further analysis using phylogenetic relationship showed that the isolate closely related with *C. kusanoi* with a bootstrap value of 99%. Isolate ClD1 was related to *Daldinia eschscholzii*, based on a BLAST search and phylogenetic analysis.

3.2. Inhibition test on growth of histamine-producing bacteria

Direct antimicrobial testing of endophytic fungi activity against *M. morganii* FNCC 0122, using the dual culture method, showed that five isolates, *Arthrobotrys foliicola* (ClA3), *C. kusanoi* (ClI1), *Fusarium proliferatum* (ClBt1), *Fusarium verticillioides* (ClBn2), and *P. ammophilae* (ClR1), all had some ability to inhibit bacterial growth, as indicated by a clear zone developing around the pieces of the fungal isolates being tested (Table 2). The five isolates obtained from root, rhizome, flower, inflorescence, or stem showed that the endophytic fungi involved were able to control *M. morganii* FNCC 0122. From the results, it was also observed that fungal isolates of the same species, but originating from different organs showed different degree of antimicrobial activity and effectiveness. This phenomenon was seen with *F. proliferatum* ClBt1 isolated from the stems, which showed antimicrobial activity; whereas, *F. proliferatum* ClI2 isolated from the inflorescence did not show any antimicrobial activity.

Further test of the selected isolates showed that only the *A. foliicola* and *F. verticillioides* water fractions of the filtrate produced positive (growth inhibition) results vis-à-vis *M. morganii* in antimicrobial tests, whereas the mycelial extract had negative results for all extracts (Table 3). Other isolates did not show any inhibitive action in respect of bacterial growth in water fractions, even though the isolates had shown inhibitive activity in direct antimicrobial screening. The results suggested that the metabolites obtained from fungal water fraction were not affected by which plant organ the endophytes originated from. The *A. foliicola* isolate was obtained from the root and *F. verticillioides* from the flower. However, the water fraction of *F. verticillioides* showed greater ability than that of *A. foliicola* to inhibit *M. morganii* growth (Table 3). The results showed that only water fractions from culture filtrate had positive results, which might indicate that the secondary metabolites produced by the endophytic fungi were extracellular.

3.3. Histamine forming inhibition test in fresh fish

Observations of water fractions of *F. verticillioides* and *A. foliicola* showed that both fractions were able to inhibit the growth of histamine-producing bacteria (Table 4). The growth of these bacteria on a modified Niven medium was indicated by the development of red-purple colonies, with surrounding medium color change from yellow to red. Immersion treatment in water fraction filtrate of *F. verticillioides* and *A. foliicola* showed the same pattern with control. The increasing number of histamine-producing bacteria was in line with the raise of incubation temperature. However, differences of bacterial growth were observed between immersion treatments and control.

Immersion treatments in water fraction of *F. verticillioides* and *A. foliicola* culture filtrates showed increasing number of histamine-producing bacteria from 0°C to 4°C were not significantly different. The number of histamine-producing bacteria began to increase significantly at 29°C. Immersion treatments in water fraction of both *F. verticillioides* and *A. foliicola* culture filtrates showed the growth enhancement of histamine-producing bacterial population at 29°C more than 10 and almost 100 times from the 0°C,

Table 1. Molecular identification using ITS regions of endophytic fungi isolated from turmeric (*Curcuma longa* L.) plant

Fungal identification	Fungal code	Plant organs	No. access GenBank references	Maximum score	% Similarity	Query cover	E value
Arthrobotrys foliicola	ClA3	Root	U51954.1	859	100	100	0.0
Cochliobolus kusanoi[*]	ClI1	Inflorescence	JX406569.1	383	81	100	9E−103
Daldinia eschscholzii	ClD1	Leaf	JX139549.1	909	100	100	0.0
Fusarium oxysporum	ClR3	Rhizome	JX045827.1	924	99	100	0.0
Fusarium proliferatum	ClBt1	Stem	AB587007.1	937	99	100	0.0
Fusarium proliferatum	ClI2	Inflorescence	AB587007.1	915	100	100	0.0
Fusarium proliferatum	ClR2	Rhizome	AB587007.1	905	99	100	0.0
Fusarium solani	ClR4	Rhizome	EU314956.1	859	100	100	0.0
Fusarium verticillioides	ClBn2	Flower	HQ637284.1	904	100	100	0.0
Phaeosphaeria ammophilae	ClR1	Rhizome	GU909731.1	688	93	100	0.0
Phanerochaete chrysosporium	ClBn3	Flower	EU872426.1	941	99	100	0.0

[*] Teleomorph stage of *Drechslera kusanoi*..

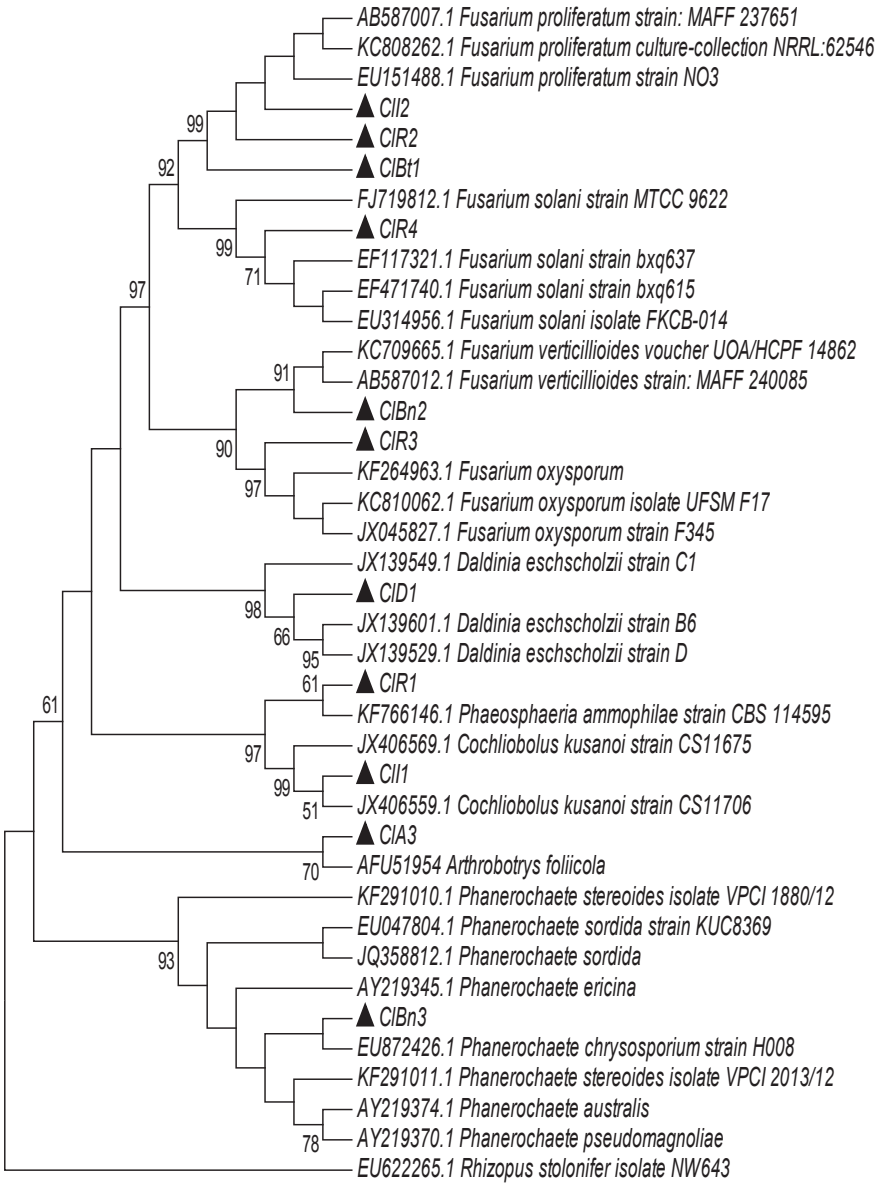


Figure. Phylogenetic tree identification results for endophytic fungi associated with turmeric plant (with a ▲ mark) based on ITS region sequences of ribosomal neighbor-joining method with Kimura 2-parameter models. Bootstrap values are indicated on the branches obtained from 1000 replications. *Rhizopus stolonifer* isolate NW643 (EU622265.1) was used as an outgroup.

Table 2. Screening of antimicrobial activity against *Morganella morganii* FNCC 0122 using dual culture method after 24 hours incubation

Endophytic fungi	Fungal code	Plant organs	Antibacterial activity
Arthrobotrys foliicola	ClA3	Root	+
Cochliobolus kusanoi	ClI1	Inflorescence	+
Daldinia eschscholzii	ClD1	Leaf	–
Fusarium oxysporum	ClR3	Rhizome	–
Fusarium proliferatum	ClBt1	Stem	+
Fusarium proliferatum	ClI2	Inflorescence	–
Fusarium proliferatum	ClR2	Rhizome	–
Fusarium solani	ClR4	Rhizome	–
Fusarium verticillioides	ClBn2	Flower	+
Phaeosphaeria ammophilae	ClR1	Rhizome	+
Phanerochaete chrysosporium	ClBn3	Flower	–

(+) Had antibacterial activity against *M. morganii* FNCC 0122; (–) had no antibacterial activity against *M. morganii* FNCC 0122.

respectively. The population of histamine-producing bacteria in the control began to increase significantly at 4°C and continued to 29°C; at that temperature, the population of histamine-producing bacteria was 100 times that at 0°C (Table 4).

In addition, interaction between immersion and storage temperature treatments appeared significantly to affect the number of histamine-producing bacteria in fresh tuna fish (Table 4). In general, the efficacy of endophytic fungi filtrate water fractions in reducing histamine-producing bacteria was affected by temperature treatments. The endophytic fungi filtrate water fractions reduced the histamine-producing bacterial population, and the reduction was statistically significant at 4°C for both fungal treatments; whereas at 29°C, a significant population reduction was only observed in the *F. verticillioides* treated culture.

Inhibition of histamine formation on fresh tuna fish using water fraction of *F. verticillioides* and *A. foliicola* culture filtrates showed that both fungal fractions were able to inhibit the formation of

Table 3. The diameter of inhibition zone (mm) of endophytic fungi water fraction against *Morganella morganii* FNCC 0122 using paper disk method after 24-hours incubation

Endophytic fungi	Diameter of inhibition (mm) at several concentrations in ppm							
	10,000		20,000		40,000		Sterile water	Chloramphenicol 32 ppm
	Filtrate	Biomass	Filtrate	Biomass	Filtrate	Biomass		
Arthrobotrys foliicola (ClA3)	6.5	0	7.7	0	8	0	0	12.3
Fusarium proliferatum (ClBt1)	0	0	0	0	0	0	0	12.3
Fusarium verticillioides (ClBn2)	7	0	8.3	0	9.3	0	0	12.3
Phanerochaete chrysosporium (ClI1)	0	0	0	0	0	0	0	12.3
Phaeosphaeria ammophilae (ClR1)	0	0	0	0	0	0	0	12.3

Table 4. The total plate count (TPC) log value of histamine-producing bacteria from tuna fillet after treatments (interaction between treatments and storage temperatures) after 24-hour incubation

Treatment	Log TPC value of histamine-producing bacteria and histamine level (ppm) at storage temperatures in °C		
	0	4	29
	TPC log		
Arthrobotrys foliicola (ClA3)	2.11^{a}	2.20^{a}	3.90^{d}
Fusarium verticillioides (ClBn2)	2.00^{a}	2.18^{a}	3.11^{c}
Control	2.15^{a}	2.78^{b}	4.00^{d}
	Histamine		
Arthrobotrys foliicola (ClA3)	3.22^{b}	8.31^{e}	124.71^{h}
Fusarium verticillioides (ClBn2)	3.12^{a}	7.98^{d}	83.20^{g}
Control	3.30^{c}	8.68^{f}	149.73^{i}

The numbers in each row and column followed by the same letter are not significantly different at 0.05 level (Duncan's multiple range test) in each group.

histamine on fresh tuna fish (Table 4). Inhibition of histamine formation in fresh tuna fish followed a similar pattern to inhibition of histamine-producing bacteria: the higher the storage temperature, the higher the level of histamine formed. The pattern of histamine formation was also in line with the size of histamine-producing bacterial population: the higher the population of histamine-producing bacteria, the higher the level of histamine formed. For the water fraction immersion treatment of *F. verticillioides* filtrate, the increasing levels of histamine at 4°C and 29°C, respectively, were significant. This pattern could also be observed in the sample subjected to immersion treatment in a water fraction of *A. foliicola* filtrate, and in the control. The amount of histamine produced at 4°C was almost three times the levels at 0°C for both treated samples, and for the control. The most significant result was seen at 29°C, where histamine levels were more than 25 times those at 0°C and more than 10 times those at 4°C for both treated samples, and the control (Table 4).

At each incubation temperature, the levels of histamine in samples treated by water fractions of two species of endophytic fungal filtrates were lower than in the control. At 0°C, the level of histamine in both fungal treatments and the control were significantly different. This pattern was observed at 4°C and 29°C. At all incubation temperatures, the samples subjected to immersion treatment in a water fraction of *F. verticillioides* filtrate, showed lower levels of histamine (Table 4), in comparison with the *A. foliicola* and control treatments. In addition, for a given immersion treatment, the storage temperature significantly affected histamine formation in fresh tuna fish.

4. Discussion

4.1. Endophytic fungi associated with the turmeric plant

Molecular analysis was used to support morphological characteristic observation. The 11 isolates obtained from turmeric plants belonged to the phylum *Ascomycota*, and *Fusarium* was the most common genus isolated. The results from the present study were similar to those reported by Xu *et al.* (2007) in a study which also found that *Fusarium* was the most common endophytic fungus found in the rhizome of the Chinese medicinal plant, *Dioscorea zingiberensis*. The number of endophytic fungi recovered from the turmeric plant was lower than the number found by Ginting *et al.* (2013), who reported 30 fungal isolates from all parts of red ginger plants.

The endophytic fungi isolated from the turmeric plant in this study had also been reported as endophytes in several other host plants. *F. proliferatum* had been reported as an endophytic fungus found on the bark and leaves of the Anglojap yew plant (*Taxus x media*), the hybrid of *Taxus baccata* and *Taxus cuspidata* (Xiong *et al.* 2013), whereas *Fusarium solani* had been found on the root and stem of the medicinal plant, *Panax ginseng*, and *Fusarium oxysporum* found on the leaves of *Nothapodytes foetida* (Park *et al.* 2012; Musavi and Balakrishnan 2013). The ClBn2 endophytic fungal isolate identified as *F. verticillioides*, was an endophyte isolated from turmeric flowers. Angelini *et al.* (2012) reported that endophytic *F. verticillioides* had been found from leaves and roots of the common reed (*Phragmites australis*). *D. eschscholzii* was found on the leaf of turmeric in this study, and had been found on leaves of the *Pholidota pallid* orchid (Sawmya *et al.* 2013).

A. foliicola was isolated from the roots of the turmeric plant and had been previously isolated from the leaf blade of the paddy plant (Zakaria *et al.* 2010). This species belongs to the group of nematodes-predator (nematophagous) fungi. Consequently, these fungi are generally found in plant organs, such as roots, which are in or near the soil; generally, colonizing only in the root epidermal and cortical cells of the host, and not penetrating vascular tissues (Lopez-Llorca *et al.* 2006). Other fungi which were isolated from the turmeric plant were *P. chrysosporium* and *P. ammophilae*. *P. chrysosporium* was isolated from the flower of *C. longa*, and has been reported as an endophyte of the *Vitis labrusca* leaf (Brum *et al.* 2012). *P. ammophilae* was isolated from the rhizome of *C. longa* and has been reported as an endophyte of the pitcher organ of *Sarracenia oreophila* (Glenn and Bodri 2012).

4.2. Histamine-producing bacterial growth inhibition test

The ability to inhibit growth of histamine-producing bacteria was not affected by the actual fungal species involved. The *F. proliferatum* ClBt1 inhibited growth of *M. morganii*, whereas the *F. proliferatum* ClI2 isolate did not show the same antibacterial activity. These results were similar to those in a study by Cui *et al.* (2011), which reported that *F. solani* YNAS09 inhibited the growth of *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*, whereas *F. solani* HNAS07 did not inhibit the growth of these three bacterial species, even though both *F. solani* isolates were obtained from the stem tissues of *Aquilaria sinensis*.

Further analysis using disc diffusion method showed that only two isolates had the ability to inhibit the bacterial growth in question, these being *A. foliicola* and *F. verticillioides*. *Arthrobotrys*

was reported to have antimicrobial activity against *Bacillus brevis* (Anke *et al.* 1995), whereas *F. verticillioides*, which had been isolated from the leaves of both *Eugenia bimarginata* and *Alchornea castaneifolia*, evidenced antimicrobial activity against *Cryptococcus neoformans* and *Bacillus cereus* (Vaz *et al.* 2012). Furthermore, positive results using the disc-diffusion method to test for inhibition of the growth of *M. morganii* were only obtained from a broth culture, which indicated that the bioactive compounds responsible for inhibiting the growth of *M. morganii* were extracellular. A study by Abdel-Motaal *et al.* (2010) reported that extracellular fractions of secondary metabolites produced by the endophytic fungi *Alternaria alternata*, *Aspergillus fumigatus*, *Drechslera hawaiiensis*, *F. solani*, *Penicillium citrinum*, *Neoscytalidium dimidiatum*, *Thyrostromella myriana*, and *Ulocladium chartarum* were more effective against the plant pathogenic fungi *Gibberella zeae* and *Thanatephorus cucumeris* than intracellular secondary metabolites.

The difference in the number of endophytic fungal species to inhibit the growth of *M. morganii* in direct dual culture and disc-diffusion method might be due to the difference of antibacterial activity because of the physiological status and environmental condition (Pereira *et al.* 2013). The difference in antibacterial activity between direct mycelia contact and mycelial extract application could also reflect differences in chemical composition themselves reflecting the diversity of metabolites (Astuti *et al.* 2014). In addition, filtrate of selected endophytic fungi were extracted with water, which process can only obtain less active compounds or achieve only partial extraction of bioactive compounds, relative to other organic solvents (Abu-Shanab *et al.* 2004; Pavithra *et al.* 2012).

Because histamine production in fish was influenced by histamine-producing bacteria, prevention strategies to suppress the bacterial growth are important. To our knowledge, this study involved the first attempt at using endophytic fungi to inhibit the growth of histamine-producing bacteria, *M. morganii*. The use of a natural product to inhibit the growth of histamine-producing bacteria was made by Paramasivam *et al.* (2007), who reported that a 5% concentration of turmeric extracts could inhibit the growth of histamine-producing bacteria, such as *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, *B. cereus*, and *Proteus mirabilis*. In this subsequent experiment, tuna fish fillets immersed in water fractions of the fungal endophyte had lower the numbers of *M. morganii* than those immersed in water only, as a control. A similar observation was reported by Setha *et al.* (2014), wherein it was noted that water does not contain bioactive compounds that can act as antimicrobial agents capable of inhabiting the growth of histamine-producing bacteria.

4.3. Histamine-forming inhibition test in fresh fish

Formation of histamine in fish is influenced by environmental conditions, histidine content, and the presence of histamine-producing bacteria (Rossano *et al.* 2006). In our study, the presence of histamine-producing bacteria and histamine production in fish fillet were measured. The presence of histamine-producing bacteria increased histamine level produced in fish fillets. The temperature also affected the growth of histamine-producing bacteria. Higher storage temperatures will cause increases in the number of bacteria. In this experiment, an ambient storage temperature of 29°C produced the highest number of histamine-producing bacteria in fish fillets. The storage condition at a given ambient temperature can also affect bacterial growth in fish (Setha *et al.* 2014). Our study showed that until ambient temperature, the water fraction of *F. verticillioides* filtrate could inhibit the forming of histamine in tuna fish fillets to acceptable levels of histamine based on Indonesian regulations.

Tuna fish fillets immersed in water fractions of endophytic fungi showed lower histamine level compared with the control treatment. A study of inhibiting histamine-forming had been performed by Setha *et al.* (2014), who reported that a water extract of *Jatropha curcas* leaves was found to inhibit histamine formation in tuna fish. Our experiment showed that histamine levels in tuna fish after immersion in filtrate water fractions of two different endophytic fungi were 124.71 and 83.20 ppm (Table 4). The levels were higher than those in the study by Setha *et al.* (2014), which reported that tuna fish immersion-treated in a water extract of *J. curcas* leaves, and then incubated at an ambient temperature for 5 hours, showed a histamine level of 17.73 ppm. It is felt that our experiment has advantages over the previous study; it used a longer incubation period at ambient temperature, and thus has better application for longer shelf-life. In addition, the materials in the later study come from turmeric plants, which are edible and commonly use as biopreservatives, whereas *J. curcas* leaves are not edible. The uses of water extracts for food processing were considered more secure than using other solvents for extraction. All in all, the ability of water fractions of endophytic fungi to inhibit the growth of histamine-producing bacteria and also histamine production, would seem to provide an important opportunity for developing natural products that reduce the levels of histamine through inhibition of histamine-producing bacteria.

The Food and Drug Administration establishes that for tuna fish, a histamine level of 50 ppm is the warning level, and that at 500 ppm, the histamine can cause poisoning (FDA 2011). The results showed that all fillets at the 29°C storage temperature had histamine levels above 50 ppm. However, Indonesian regulations for the histamine level in fresh tuna fish required that this be no higher than 100 ppm (BSN 2006). Therefore, based on the present study, only the tuna fillet, which was immersed in the *F. verticillioides* filtrate water fraction, and which showed an 83.2 ppm histamine level, can be consumed. This result showed that the *F. verticillioides* filtrate water fraction was suitable for tuna fish storage at ambient temperature.

The two isolates which showed antimicrobial activity and inhibition of histamine formation were not derived from the rhizome, but rather from the roots and flowers. This is interesting because the rhizome of the turmeric plant is generally used for medicinal purposes. Therefore, the exploration of other turmeric organs as potential source for further drug discovery should be carried out. Furthermore, based on this study, endophytic fungal extracts could be used as an alternative solution for biopreservative purpose replacing synthetic chemical compounds that might be harmful for human health and the environment.

Conflict of interest

The Authors declare no conflict of interest.

Acknowledgment

The authors are grateful to Mark Reynolds, ARCh Language Training, ILP Cimanggu-Bogor, Indonesia, and Dr Margaret Cargill, Adelaide University, Australia for English language editing.

References

Abdel-Motaal FF, Nassar MSM, El-Zayat SA, El-Sayed MA, Ito SI. 2010. Antifungal activity of endophytic fungi isolated from Egyptian henbane (*Hyoscyamus muticus* L.). *Pak J Bot* 42:2883–94.

Abu-Shanab B, Adwan G, Abu-Safiya D, Jarrar N, Adwan K. 2004. Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. *Turk J Biol* 28:99–102.

Akter T, Ahmed ATA, Khaleque MA, Begum M. 2013. Effect of drying on quality of tengra (*Mystus vittatus*) treated with turmeric and salt. *Unique Res J Biol Sci* 1:1–5.
Angelini P, Rubini A, Gigante D, Reale L, Pagiotti R, Venanzoni R. 2012. The endophytic fungal communities associated with the leaves and roots of the common reed (*Phragmites australis*) in Lake Trasimeno (Perugia, Italy) in declining and healthy stands. *Fungal Ecol* 5:683–93.
Anke H, Stadler M, Mayer A, Sterner O. 1995. Secondary metabolites with nematicidal and antimicrobial activity from nematophagous fungi and Ascomycetes. *Can J Bot* 73:932–9.
Astuti P, Sudarsono S, Nisak K, Nugroho GW. 2014. Endophytic fungi isolated from *Coleus amboinicus* exhibited antimicrobial activity. *Adv Pharm Bull* 4:599–605.
Barnett HL, Hunter BB. 1998. Illustrated Genera of Imperfect Fungi, 4th edition. St. Paul: Prentice-Hall, Inc, St. Paul.
Barrow JR, Lucero ME, Reyes-Vera I, Havstad KM. 2008. Do symbiotic microbes have a role in plant evolution, performance and response to stress? *Commun Integr Biol* 1:69–73.
Bjornsdottir K, Bolton GE, McClellan-Green PD, Jaykus LA, Green DP. 2009. Detection of gram-negative histamine-producing bacteria in fish: a comparative study. *J Food Prot* 72:1987–91.
Brum MCP, Araujo WL, Maki CS, Azevedo JL. 2012. Endophytic fungi from *Vitis labrusca* L. (Niagara Rosada) and its potential for the biological control of *Fusarium oxysporum*. *Genet Mol Res* 11:4187–97.
BSN National Standardization Agency. Indonesian National Standard 01-2729.1-2006. Fresh Fish. Chapter 1: Specification. Jakarta: National Standarization Agency.
Cui JL, Guo SX, Xiao PG. 2011. Antitumor and antimicrobial activities of endophytic fungi from medicinal parts of *Aquilaria sinensis*. *J Zhejiang Univ SCI B* 12:385–92.
Dai CC, Yu BY, Li X. 2008. Screening of endophytic fungi that promote the growth of *Euphorbia pekinensis*. *Afr J Biotechnol* 7:3505–9.
FDA Food and Drug Administration. 2011. Fish and Fishery Products Hazards and Controls Guidance, 4th edition. Washington DC: Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington DC.
Ginting RCB, Sukarno N, Widyastuti U, Rusman LK, Kanaya S. 2013. Diversity of endophytic fungi from red ginger (*Zingiber officinale* Rosc.) plant and their inhibitory effect to *Fusarium oxysporum* plant pathogenic fungi. *HAYATI J Biosci* 20:127–37.
Glenn A, Bodri MS. 2012. Fungal endophyte diversity in *Sarracenia*. *PLoS One* 7, e32980.
Hallmann J, Berg G, Schulz B. 2006. Isolation procedures for endophytic microorganisms. In: Schulz B, Boyle C, Sieber TN (Eds.). *Soil Biology*, Vol. 9. Springer-Verlag Berlin Heidelberg.
Lopez-Llorca LV, Jansson H-B, Vicente JGM, Salinas J. 2006. Nematophagus fungi as root endophytes. In: Schulz BJE, Boyle CJC, Sieber TN (Eds.). *Microbial Root Endophytes*. Berlin (GER): Springer-Verlag.
Musavi SF, Balakrishnan RM. 2013. Biodiversity, antimicrobial potential, and phylogenetic placement of an endophytic *Fusarium oxysporum* NFX 06 isolated from *Nothapodytes foetida*. *J Mycol* 2013:1–10.
Paramasivam S, Thangaradjou T, Kannan L. 2007. Effect of natural preservatives on the growth of histamine-producing bacteria. *J Environ Biol* 28:271–4.
Park YH, Lee SG, Ahn DJ, Kwon TR, Park SU, Lim HS, Bae H. 2012. Diversity of fungal endophytes in various tissues of *Panax ginseng* Meyer cultivated in Korea. *J Ginseng Res* 36:211–7.
Pavithra N, Sathish L, Ananda K. 2012. Antimicrobial and enzyme activity of endophytic fungi isolated from Tulsi. *J Pharm Biomed Sci* 16:1–6.
Pereira E, Santos A, Reis F, Tavares RM, Baptista P, Lino-Neto T, Almeida-Aguiar C. 2013. A new effective assay to detect antimicrobial activity of filamentous fungi. *Microbiol Res* 168:1–5.
Rossano R, Mastrangelo L, Ungaro N, Riccio P. 2006. Influence of storage temperature and freezing time on histamine level in the European anchovy *Engraulis encrasicholus* (L., 1758): a study by capillary electrophoresis. [short communication]. *J Chromatogr B* 830:161–4.
Sambrook J, Russell DW. 2001. Molecular Cloning: A Laboratory Manual, 3rd edition. New York: Cold Spring Harbor Laboratory Pr, New York.
Sawmya K, Vasudevan TG, Murali TS. 2013. Fungal endophytes from two orchid species-pointer towards organ specificity. *Czech Mycol* 65:89–101.
Setha B, Laga A, Mahendradatta M, Firdaus. 2014. Inhibition of histamine formation on the frigate tuna (*Auxis thazard thazard*, L) using leaf extract of *Jatropha curcas*. *Int J Adv Res* 2:350–6.
Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–9.
Vaz ABM, Brandao LR, Vieira MLA, Pimenta RS, Morais PB, Sobral MEG, Rosa LH, Rosa CA. 2012. Diversity and antimicrobial activity of fungal endophyte communities associated with plants of Brazilian savanna ecosystems. *Afr J Microbiol Res* 6:3173–85.
White TJ, Bruce T, Lee S, Taylor I. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds.). *PCR Protocols: A Guide to Method and Applications*. New York: Academic Pr, New York.
Wohrl S, Hemmer W, Focke M, Rappersberger K, Jarisch R. 2004. Histamine intolerance-like symptoms in healthy volunteers after oral provocation with liquid histamine. *Allergy Asthma Proc* 25:305–11.
Wortberg B, Gerd Z. 1981. Zum nachweis von histamine neben tyramin, putrescin und cadaverin in lebensmitteln. *Lebensmittel Chem Gericht Chem* 35:89–92.
Xiong ZQ, Yang YY, Zhao N, Wang Y. 2013. Diversity of endophytic fungi and screening of fungal paclitaxel producer from Anglojap yew, *Taxus x media*. *BMC Microbiol* 13:1–10.
Xu L, Zhou L, Zhao J, Li J, Li X, Wang J. 2007. Fungal endophytes from *Dioscorea zingiberensis* rhizomes and their antibacterial activity. *Lett Appl Microbiol* 46: 68–72.
Zakaria L, Yaakop AS, Salleh B, Zakaria M. 2010. Endophtyic fungi from paddy. [short communication]. *Trop Life Sci Res* 21:101–7.
Zhang Y, Mu J, Feng Y, Kang Y, Zhang J, Gu PJ, Wang Y, Ma LF, Zhu YH. 2009. Broad-spectrum microbial epiphytic and endophytic fungi from marine organisms: isolation, bioassay and taxonomy. *Mar Drugs* 7:97–112.
Zhao J, Shan T, Mou Y, Zhou L. 2011. Plant-derived bioactive compounds produced by endophytic fungi. *Mini Rev Med Chem* 11:159–68.